The first minutes in the life of a peroxisomal matrix protein

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Running title: Protein sorting to the peroxisome matrix

Highlights:

- The vast majority of peroxisomal matrix proteins are homo-oligomers.
- How these proteins are sorted to the organelle has been a matter of debate.
- We argue that this sorting pathway is best explained by a monomer-based import mechanism.

Abbreviations:

ACOX, acyl-CoA oxidase; AO, alcohol oxidase; CCS, copper chaperone of SOD1; DTM, docking/translocation machinery; Gpd1p, glycerol-3-phosphate dehydrogenase 1; LDHA/B/Bx, lactate dehydrogenase isoforms A, B and B readthrough-extended, respectively; PIM, peroxisomal import machinery; Pnc1p, nicotinamidase; PTS, protein targeting signal; SOD1, Cu/Zn superoxide dismutase; TPR, tetratricopeptide repeat; UOX, urate oxidase.

Abstract

In the field of intracellular protein sorting, peroxisomes are most famous by their capacity to import oligomeric proteins. The data supporting this remarkable property are abundant and, understandably, have inspired a variety of hypothetical models on how newly synthesized (cytosolic) proteins reach the peroxisome matrix. However, there is also accumulating evidence suggesting that many peroxisomal oligomeric proteins actually arrive at the peroxisome still as monomers. In support of this idea, recent data suggest that PEX5, the shuttling receptor for peroxisomal matrix proteins, is also a chaperone/holdase, binding newly synthesized peroxisomal proteins in the cytosol and blocking their oligomerization. Here we review the data behind these two different perspectives and discuss their mechanistic implications on this protein sorting pathway.

Keywords:
Protein translocation, peroxisome, PEX5, PEX7, PTS1, PTS2

“No part of the world can simply be read - it always must be interpreted, and those interpretations are subject to constant reevaluation.”
Mark Ptashne [1]

1. Introduction

Peroxisomes are round-shaped organelles delimited by a single membrane. Their size, number and protein repertoire varies widely among organisms, cell types and even physiological conditions [2]. In mammals, peroxisomes have a relatively simple composition comprising about 100 different proteins [3,4]. Despite this simplicity, peroxisomes are involved in important metabolic pathways and, accordingly, mutations in genes encoding peroxisomal enzymes, peroxisomal membrane metabolite transporters, or proteins involved in peroxisome biogenesis cause devastating diseases in humans [5,6].

All peroxisomal matrix proteins are synthesized in the cytosol and post-translationally transported to the organelle [7]. Their specific sorting to this compartment is mediated by a complex machinery comprising a core of 10 evolutionary conserved peroxins (peroxins are proteins specifically involved in peroxisomal biogenesis) plus a set of additional proteins most of which are involved in ubiquitin conjugation and deconjugation ([8–13]; see also Table I in ref. [14], this issue).

In order to be sorted to the peroxisome matrix, a newly synthesized protein must have a peroxisomal targeting signal (PTS) in its polypeptide chain. There are two well-characterized types of PTSs: the PTS type 1 (PTS1), the most common, is a small peptide present at the C termini of proteins and frequently ends with the sequence S-K-L [15]; the PTS2 is a degenerated nonapeptide found at the N termini of a few peroxisomal proteins [16,17]. Unlike the PTS1, which is not processed upon import, the PTS2 is generally cleaved when the protein reaches the peroxisome matrix of higher eukaryotes [17].

In mammals, plants and many other organisms, sorting of both PTS1- and PTS2-containing proteins requires PEX5 [18–22], a monomeric protein of about 70 kDa possessing an intrinsically disordered N-terminal half and a globular C-terminal half comprising six tetratricopeptide repeat motifs (hereafter simply referred to as TPRs) [23–26]. Our knowledge on how PEX5 interacts with these two types of cargoes is still fragmented. Structural and protein-protein interaction studies have shown that the PTS1 signal interacts with the TPRs of PEX5 [25,27–29]. However, it is now clear that the PEX5-cargo protein interaction is not limited to this binding interface and that the N-terminal half of PEX5 and other regions of the PTS1 cargo proteins are also involved [30–34]. Much less is known on the PEX5-PTS2 cargo protein interaction. Actually, until recently, it was frequently considered that the PEX5-PTS2 interaction might even not be

direct but rather bridged by PEX7, a WD-repeat protein long-known to interact with both the PTS2 peptide and a small domain present in the N-terminal half of PEX5 [18–22,35]. However, recent structural data of a trimeric protein complex comprising yeast PEX7, an artificial PTS2 protein and a small fragment of PEX21 (the yeast orthologue of mammalian/plant PEX5 in the PTS2-mediated protein import pathway; [36,37]), revealed that PEX21 also interacts directly with the PTS2 peptide [38]. Given the functional and structural similarities between yeast PEX21 and the N-terminal half of mammalian/plant PEX5 [36,37], it is therefore likely that the same is valid for mammalian/plant PEX5. In agreement with this possibility, two recent studies have shown that the human PEX7.PTS2 interaction is drastically stabilized by PEX5 [39,40].

One of the most important properties of PEX5 and PEX7 regards their intracellular localization. Indeed, in contrast to all the other peroxins involved in this protein sorting pathway, which are peroxisomal proteins, pioneering studies on PEX5 and PEX7 revealed that both display a dual subcellular localization, cytosolic and peroxisomal [41,42]. This property, together with their capacity to bind PTS1 and PTS2 proteins, is at the basis of a central concept found in all mechanistic models published to date, namely, that newly synthesized peroxisomal matrix proteins are recognized in the cytosol and transported to the organelle by the shuttling receptors PEX5/PEX7; after delivering their cargoes into the organelle, the receptors return to the cytosol to promote additional rounds of protein transport [41,42].

Another important early discovery that has heavily influenced models on the mechanism of protein transport to the peroxisome matrix was the observation by several researchers that these organelles can acquire already oligomerized proteins from the cytosol (see Section 3.). Two main models were then proposed to explain this remarkable capacity of peroxisomes [43]. One, a translocation-based model, postulated the existence of large regulated channels/pores at the peroxisomal membrane; the other, proposed that large already-oligomerized cargo proteins might reach the peroxisomal matrix by an endocytosis-like mechanism. Data clearly favoring the translocation-based model came a few years later from the biochemical characterization of peroxisome-associated PEX5. Indeed, it was found that during its transient passage through the peroxisome, PEX5 acquires a transmembrane topology, exposing a small N-terminal domain into the cytosol whereas the bulky part of its polypeptide chain faces the organelle matrix [44,45]. Since the main cargo protein-binding domain of PEX5, the TPRs, resides at its C terminus and occupies about half of PEX5 polypeptide chain, this finding immediately suggested that cargo proteins are translocated across the organelle membrane by PEX5 itself when the receptor becomes inserted into a transmembrane protein complex of the peroxisome, the docking/translocation machinery (DTM)[44,46]. Subsequent characterization of the insertion of PEX5 into the DTM revealed that this

step is a cargo-dependent but ATP-independent process, strongly suggesting that the driving force for protein translocation across the organelle membrane derives from strong protein-protein interactions involving PEX5 on one side and peroxins of the DTM on the other [46–48].

Although it is now generally accepted that peroxisomal proteins reach the organelle matrix using a translocation-based mechanism [8–10,49–51], there are still many questions (and disputes among researchers in the field) on the architecture and mechanism of the machinery that accomplishes this task. One of these questions regards precisely one of the most famous properties of peroxisomes, *i.e.*, their capacity to import already oligomerized proteins. Although the data supporting this property are abundant, several findings made over the years suggest that import of already oligomerized proteins may not be that frequent and that many of these proteins may actually arrive at the organelle still as monomers.

Here we summarize and discuss the main data behind these two different perspectives. As it will be apparent below, many of the points we raise argue against an oligomeric protein import model favoring instead a monomeric protein import model. This is not to say that the oligomeric protein import model is not valid at all. Actually, for a few components of the peroxisome, the oligomeric protein import model still provides the best explanation for their presence in the organelle. Ultimately, our goal is to stimulate research on this topic so that the peroxisomal protein import machinery stops being one of the least understood protein import machineries of the eukaryotic cell.

2. Peroxisomal matrix proteins: the first events after synthesis

As stated in the previous section, peroxisomal matrix proteins are synthesized on soluble cytosolic ribosomes [7]. Thus, as with many other proteins that do not follow the secretory pathway, folding of their polypeptide chains is catalyzed by the cytosolic chaperone machinery and probably starts as soon as the first N-terminal amino acid residues emerge from the ribosomal polypeptide exit tunnel [52–58]. What happens to these proteins in the first seconds after folding has not been explored in detail. Nevertheless, it is reasonable to assume that peroxisomal matrix proteins that are monomers in their native state are simply recognized by cytosolic PEX5/PEX7 and transported to the organelle. However, for proteins that are homo-oligomers in their native conformation, the pathway may be different, as explained below.

An interesting property of several peroxisomal homo-oligomeric proteins regards the fact that they can be detected as soluble monomeric proteins immediately after synthesis, both *in vivo* [59–61] and *in vitro* [34,62,63]. This behavior suggests, on one hand, that these monomers are already at least partially folded and, on the other, that folding of monomers and their oligomerization to yield the native enzymes are not

physically coupled events. The same conclusion is probably valid for monomer folding and monomer-PEX5 interaction because some active oligomeric peroxisomal proteins can be detected in the cytosol of cells lacking PEX5 [64–66] and the *in vitro* protein synthesis system used in the experiments referred to above has essentially no endogenous PEX5 [34,62,63]. Thus, it appears that newly synthesized proteins are released by the cytosolic chaperone machinery as soluble monomeric proteins independently of PEX5. An obvious implication of this reasoning is that all subsequent protein-protein interactions occurring in the cytosol are probably of stochastic nature. If so, one can consider two possible pathways for these proteins: 1) interaction with PEX5/PEX7 and/or 2) oligomerization. The first pathway leads us to a "monomeric protein import model" whereas the second is the basis of an "oligomeric protein import model" (see Figure 1). Determining which pathway prevails is not a mechanistic detail of minor importance because, at the very least, it can provide us with valuable information on how the peroxisomal DTM functions.

3. Evidence for the oligomeric protein import model

The experimental evidence behind the concept that peroxisomal matrix proteins oligomerize in the cytosol before import into the organelle dates back to 1994 [43,67]. The experiments reported in those two studies consisted of expressing in the same cells two versions of a protein, which is homo-oligomeric in its native state. One version contained a PTS whereas the other lacked such a signal. Expression of the PTS-less protein alone resulted in its cytosolic localization, as expected. However, when this protein was co-expressed with the PTS-containing version, the two proteins were now found in the peroxisome. Apparently, the protein lacking the PTS was transported to the organelle piggy-backed with its PTS-containing partner. Similar findings were subsequently reported for other peroxisomal oligomeric proteins in several organisms/cell lines (see Table 1). There are three aspects of those experiments that deserve discussion. First, with only a few exceptions (see Section 6.), all these studies used experimental conditions that lead to very high levels of the characterized proteins. As discussed recently, these conditions can potentially lead to the titration of the peroxisomal protein import machinery (PIM), and thus to the ectopic (*i.e.*, cytosolic) oligomerization of the reporter proteins [63]. Second, in only one case was the import kinetics of the reporter oligomeric protein documented [43]. Significantly, the authors found that in contrast to endogenous peroxisomal proteins, which display half-lives of import of a few minutes, import of the oligomeric reporter protein occurred over a period of many hours. From the several possibilities that were considered to explain this finding, one was that the import of oligomeric proteins may be an intrinsically low efficiency process [43]. Finally, by co-expressing a PTS-containing protein together with

its PTS-less version, these experiments create an artificial situation in which a protein destined to the peroxisome, and that, therefore, should interact only with its receptor, can now also interact with a protein that is not recognized by PEX5/PEX7, and thus be rerouted into a non-natural pathway. Although a situation of this type may actually occur in a few cases (see Section 6.), most, if not all, newly synthesized subunits of oligomeric peroxisomal matrix proteins have PTSs, and thus they all can potentially interact with cytosolic PEX5/PEX7.

In addition to the co-expression experiments described above, two pulse-chase analyses in yeasts were reported, both also pointing to the idea that some peroxisomal proteins (but not all; see Section 4.) arrive at the peroxisome after oligomerization in the cytosol [68,69]. Indeed, at short times after pulse-labeling the cytosolic pools of both *Candida boidinii* dihydroxyacetone synthase and *Yarrowia lipolytica* acyl-Coa oxidase (ACOX) were found to be already in their oligomeric forms. These pools decreased during subsequent chase incubations with the concomitant appearance of the two proteins in organelle fractions. Although these pulse-chase approaches are much more elegant than the co-expression experiments, it should be noted that the experimental conditions used in those studies also lead to a drastic proliferation of peroxisomes, and thus the criticism raised above regarding a potential titration of the PIM also applies here. Another issue with these experiments regards the fact that it is difficult to exclude the possibility that oligomerization of the proteins studied occurred, not *in vivo*, during the pulse-chase labeling, but rather during processing of the samples for biochemical analyses.

Another argument that was used to support the concept that peroxisomal proteins arrive at the organelle matrix already in their oligomeric state was based on the idea that peroxisomes seemed to lack their own protein folding machinery [54,69,70]. Thus, peroxisomal proteins should first undergo folding and oligomerization in the cytosol, a subcellular compartment where chaperones are abundant, and only then be imported into the organelle. However, we now know that peroxisomes from several organisms do harbor some protein chaperone activity [71–73]. Furthermore, it is conceptually feasible that a newly synthesized protein can acquire a near native conformation in the cytosol in a chaperone-mediated process, be imported into the organelle still as a monomer and oligomerize in the peroxisomal matrix in a spontaneous, unassisted manner (see also ref.[69]).

Regardless of the criticisms that may or not be raised over the evidence supporting the oligomeric protein import model, it must be noted that all that work ended up revealing a property of the PIM of paramount importance for the comprehension of its mechanism. Indeed, the finding that peroxisomes can import already oligomerized proteins demonstrated that the peroxisomal protein import

machinery can accept already folded proteins as substrates. This property contrasts with those of other protein import machineries, such as the ones found in mitochondria or the endoplasmic reticulum, which require their substrates to be unfolded [74,75]. Clearly, the peroxisomal protein import machinery abides by different principles.

4. Evidence for the monomeric protein import model

The first evidence supporting the idea that peroxisomal proteins arrive at the organelle matrix still as monomers, come from a pulse-chase analysis of rat liver catalase, a homotetrameric protein comprising 15% of the total protein mass of liver peroxisomes [61]. The authors found that at short times after labeling, newly synthesized catalase was detected in the cytosol as a monomeric inactive protein. This pool was subsequently imported into peroxisomes (half-time of import of 14 min) where it could be found still in a monomeric state; its oligomerization occurred inside the organelle. A similar pulse-chase analysis of *C. boidinii* alcohol oxidase (AO), an octameric protein in its native state, yielded essentially the same conclusion – the protein was detected in the cytosol as a monomer and the octameric form could be detected only in peroxisomes at later time points [59,69].

In vitro import assays aiming at comparing the import efficiencies of monomeric and oligomeric versions of three different proteins also pointed into the same direction: the monomeric versions of *Cucurbita pepo* isocitrate lyase, mouse ACOX1 and urate oxidase (UOX) were found to be better import substrates than the corresponding oligomeric forms [56,63]. Actually, for ACOX1 and UOX no evidence for *in vitro* import of their oligomeric forms could be obtained [63].

Several other observations support the notion that peroxisomal proteins may reach the organelle matrix still as monomers (see Table 2). First, there is an increasing number of peroxisomal proteins which do not interact with PEX5 upon oligomerization [76–78]. The simplest way to explain their peroxisomal localization is to consider that these proteins are recognized and transported to the organelle before oligomerization (see also Section 5.). If this is so, then why should it be different for all the other oligomeric peroxisomal proteins? Some recently described properties of mammalian PEX5 suggest that it is not. First, the concentration of cytosolic PEX5 in a rat hepatocyte is similar to the sum of cytosolic concentrations of all newly synthesized peroxisomal proteins that are en route to the organelle [34]. Thus, all newly synthesized proteins could potentially interact with PEX5 shortly after their synthesis/folding. Second, it has been shown that PEX5 interacts quite efficiently with the monomeric versions of human catalase and mouse sterol carrier protein x, ACOX1 and UOX, four prominent oligomeric proteins of liver peroxisomes [34,62,63]. Actually, in the case of catalase, the interaction between its monomeric version and PEX5 seems to be much stronger than the

interaction between the tetrameric enzyme and PEX5 [34]. Finally, for the three proteins found to oligomerize *in vitro*, it was shown that binding to PEX5 and homo-oligomerization are mutually exclusive events. The latter two PEX5 properties led us to propose that PEX5, in addition to its role as a receptor and translocator for peroxisomal matrix proteins, is also a chaperone/holdase that binds newly synthesized monomeric proteins in the cytosol avoiding premature or unspecific interactions.

5. Monomeric *vs.* oligomeric protein import model

If peroxisomes can import oligomeric proteins why then should this not be the regular pathway? After all, if a single PEX5 molecule could transport an oligomeric protein to the peroxisomal matrix, instead of its subunits, one by one, such a pathway would surely save ATP to the cell (the ATP-consuming step of this protein sorting pathway resides in the extraction of the receptors from the DTM; [47,79,80]). The problem with this perspective is that it fails to provide plausible explanations for an increasing number of observations. For instance, as already stated above, it is difficult to envisage how those oligomeric proteins that no longer expose their PTSs might be imported into the peroxisome. Naturally, one could assume that there are specific cytosolic chaperones that recognize these oligomeric proteins and pull their PTSs out of their globular bodies allowing PEX5 to recognize them [81]. However, no evidence for the existence of these chaperones was ever obtained, quite the contrary. Indeed, when two of these oligomeric proteins are placed in the cytosol of cells either by exploring the properties of a yeast temperature-sensitive *pex1* mutant [78] or simply by protein transfection of mammalian cells [76], they remain in the cytosol. The monomeric protein transport model makes no such assumption. It just builds on the experimental data described above suggesting that a monomeric soluble protein harboring a PTS1 can interact with PEX5, that this interaction blocks its subsequent oligomerization and that there is sufficient PEX5 in the cell cytosol to bind all newly synthesized peroxisomal proteins.

Another difference between the two models regards the predictions they make at downstream steps, namely those that occur at the peroxisomal DTM. One model proposes that a single PEX5 molecule carrying a single cargo protein docks at and gets inserted into the peroxisomal membrane DTM, releasing the cargo protein into the organelle matrix [82]. Regardless of the cargo protein size, which may be as small as 15 kDa (*e.g.*, sterol carrier protein 2) or as large as 270 kDa (*e.g.*, xanthine oxidase), such a model predicts that a reasonably flexible DTM with a single fixed geometry could deal with all peroxisomal matrix proteins (note that for globular/spherical proteins the diameter of a 270-kDa protein is only 2.6-fold larger than that of a 15-kDa protein). In contrast, the oligomeric protein import model raises the possibility that many of the

cargoes that arrive at the DTM, do so bound to several PEX5 molecules [70,83,84]. We could assume that only one of these PEX5 molecules, the first to collide with the DTM, becomes inserted into the DTM thus pushing the oligomeric cargo protein across the peroxisomal membrane; but, if so, what happens then to the other PEX5 molecules that may remain bound to the cargo? Are they translocated into the peroxisome matrix together with the cargo? Or is there a mechanism to avoid that these PEX5 molecules enter the organelle? Alternatively, we could assume that all PEX5 molecules bound to an oligomeric cargo protein contribute to cargo translocation, *i.e.*, that they all become inserted into the DTM. This would require a DTM with the capacity to interact with PEX5-cargo protein complexes displaying rather different geometries, from a 1:1 PEX5-monomeric cargo protein complex, to linear and V-shaped PEX5-cargo-PEX5 complexes, to complexes containing three or more PEX5 molecules, some of which might not even exist in a single dimensional plane. Does such machinery exist? No answers have yet been provided for these questions.

6. Some proteins are imported as hetero-oligomers under conditions where PIM components are not limiting

The PEX5 properties described above suggest that many newly synthesized oligomeric peroxisomal proteins will remain in the monomeric state in the cytosol, as long as the amount of PEX5 does not become stoichiometrically limiting. However, the stochastic nature of the PEX5-cargo protein interaction also implies that whenever PEX5 is limiting, cytosolic folded monomers can follow a homo-oligomerization pathway, as is probably the case in experiments where the levels of some peroxisomal proteins are dramatically increased (see Section 3.). There are, however, three cases where import of oligomers occurs under conditions where there are no reasons to believe that PEX5 or other components of PIM are stoichiometrically limiting. One regards rat liver Cu/Zn superoxide dismutase 1 (SOD1) [85]; another, the two isoforms of human lactate dehydrogenase (LDH), LDHA and LDHB [86]; the third, the yeast nicotinamidase Pnc1p [87]. Indeed, none of these proteins possess a PTS. Yet, a fraction of them can be found in peroxisomes [88–91]. How do these proteins reach the organelle? For SOD1 it was shown that one of its chaperones, copper chaperone of SOD1 (CCS), does possess a PTS1 whereas for LDH it was recently found that translational readthrough of the stop codon in the *LDHB* transcript yields an extended isoform of this protein, the so-called LDHBx, which possesses a PTS1 and can form heterotetramers with both LDHA and LDHB [85,86]. Likewise, yeast Pnc1p was recently shown to interact with the PTS2-containing Gpd1p, a glycerol-3-phosphate dehydrogenase displaying a partial peroxisomal localization [87]. Apparently, all these PTS-less proteins reach the peroxisome matrix piggy-backed with the partners that do have one. Interestingly, for

the SOD1-CCS heterodimer this sorting pathway seems to be rather inefficient. Indeed, it was estimated that less than 10% of total CCS reaches the peroxisomes [85], a finding which according to those authors might reflect the existence of a weak PTS1 in CCS. An alternative explanation would be to assume that newly synthesized CCS interacts rapidly with the SOD1 protein (which lacks a PTS and therefore is not bound by PEX5) and that peroxisomal import of this heterodimer is a low efficiency process. In essence, this situation might be similar to those described in Section 3., where a PTS containing protein is co-expressed with a PTS-less partner, thus "diverting" the PTS protein (in this case, the CCS protein) from an immediate interaction with PEX5. The data for the yeast Gpd1p.Pnc1p heterodimer are more complex because the PTS2 signal of Gpd1p seems to be regulated by phosphorylation [92]. Thus, it is presently unclear whether the large extra-peroxisomal pools of both Gpd1p and Pnc1p that are detected in yeast cells under all experimental conditions tested reflect a low import efficiency of the heterodimeric complex or the phosphorylation status of Gpd1p. The cytosol/peroxisome distribution of LDHBx is unknown and thus, the import efficiency of the LDHBx-containing heterotetramers also remains undefined [86]. Regardless, these three examples show that peroxisomes can import oligomeric proteins even under conditions where PIM components are probably not stoichiometrically limiting. A main challenge now will be to determine the import kinetics of these proteins.

7. Conclusion

It is accepted that peroxisomes can import oligomeric proteins. However, the fact that they can does not imply that they generally do. Indeed, as discussed above, several observations made over the many years of research in this field are best explained by the monomeric protein import model. Nevertheless, there are also data suggesting that peroxisomes import at least minor amounts of some oligomeric proteins even under conditions where PIM components are not stoichiometrically limiting. Interestingly, the three known examples, CCS.SOD1, LDHBx.LDHB/A, and Gpd1p.Pnc1p are all hetero-oligomers in which only one of the subunits has a PTS. Thus, as with the monomers of some newly synthesized peroxisomal oligomeric proteins, they are bound and transported to the organelle by just one receptor molecule.

The jury is still out regarding the architecture of the docking/translocation machinery through which newly synthesized proteins are translocated into the peroxisome matrix. As emphasized here, a better characterization of its substrates will provide valuable information to understand how this machinery works.

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Figure legends

Figure 1. **The monomeric and oligomeric protein import models.**
Peroxisomal matrix proteins are synthesized on free cytosolic ribosomes and folded into soluble monomers by the cytosolic chaperone machinery. Proteins that are monomeric in their native state (*e.g.*, L-bifunctional protein; [93]) interact immediately with PEX5/PEX7 (PEX7 is not shown for simplicity). Proteins that are oligomeric in their native state can follow two pathways: 1) import into the peroxisome as monomers – the monomeric protein import model; or 2) oligomerization and subsequent import – the oligomeric protein import model. The experimental evidence supporting each of these models is provided in the main text. Note that oligomerization of some proteins hinders their interaction with PEX5. These proteins may only be targeted to the organelle as monomers. Also, oligomeric proteins that expose their PTSs might interact with several PEX5 molecules in the cytosol. How the docking/translocation machinery (DTM) might deal with these substrates is unknown. Folding of cofactor-containing proteins may be dependent [68] or independent [94] of the cofactor itself (*e.g.*, FAD, thiamine pyrophosphate, heme). In the latter case, cofactor incorporation may occur in the cytosol and/or peroxisome.

Table 1. Studies reporting import of peroxisomal matrix proteins as oligomers.

Table 2. Studies reporting import of peroxisomal matrix proteins as monomers.

Table 1. Studies reporting import of peroxisomal matrix proteins as oligomers.

Protein	Organism	Quaternary Structure	Refs
Chloramphenicol acetyltransferase	engineered bacterial protein	Trimeric	[43]
3-ketoacyl-CoA thiolase	*Saccharomyces cerevisiae*	Dimeric	[67]
Alanine-glyoxylate aminotransferase	*Homo sapiens*	Dimeric	[95]
Malate dehydrogenase	*Saccharomyces cerevisiae*	Dimeric	[96]
Isocitrate lyase	*Gossypium hirsutum*	Tetrameric	[97]
	Ricinus communis		
	Brassica napus		
Catalase	*Candida boidinii*	Tetrameric	[98]
	Homo sapiens	Tetrameric	[99]
Dihydroxyacetone synthase	*Candida boidinii*	Dimeric	[69]
	Hansenula polymorpha	Dimeric	[78]
3,2-trans-enoyl-CoA isomerase	*Saccharomyces cerevisiae*	Monomeric	[100]
Acyl-CoA oxidase	*Yarrowia lipolytica*	Heteropentameric	[68]
Peroxisomal membrane protein LPX1	*Saccharomyces cerevisiae*	Dimeric	[101,102]
Cu/Zn Superoxide Dismutase/ Copper chaperone of SOD1	*Rattus norvegicus*	Heterodimeric	[85]
HEX	*Neurospora crassa*	Hexameric	[83]
Lactate dehydrogenase	*Homo sapiens*	Heterotetrameric	[86]
Nicotinamidase /Glycerol-3-phosphate dehydrogenase 1	*Saccharomyces cerevisiae*	Heterodimeric	[87]

Table 2. Studies reporting import of peroxisomal matrix proteins as monomers.

Protein	Organism	Quaternary Structure	Refs
Catalase	*Rattus norvegicus*	Tetrameric	[61]
Malate synthase	*Cucumis sativus*	Octameric	[60]
Alcohol oxidase	*Candida boidinii*	Octameric	[59,69]
	Hansenula polymorpha	Octameric	[78,103,104]
	Pichia pastoris	Octameric	[105]
Isocitrate lyase	*Cucurbita pepo*	Tetrameric	[56]
Dehydrogenase/reductase SDR family member 4	*Sus scrofa*	Tetrameric	[76]
Soluble epoxide hydrolase	*Homo sapiens*	Dimeric	[77]
Acyl-CoA oxidase	*Mus musculus*	Dimeric	[63]
Urate oxidase	*Mus musculus*	Tetrameric	[63]

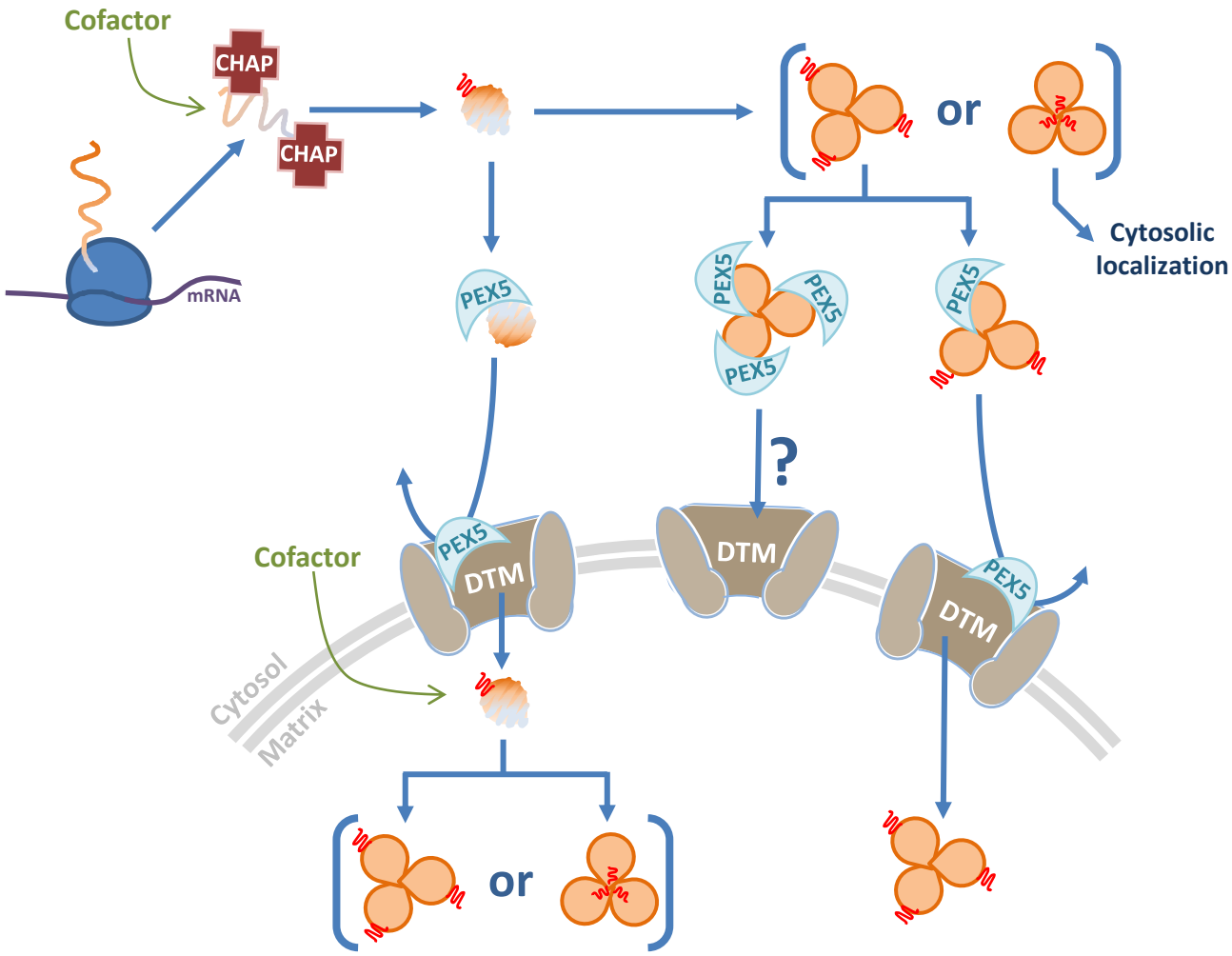
Cofactor
CHAP
CHAP
mRNA
PEX5
or
Cytosolic
localization
PEX5
PEX5
PEX5
PEX5
?
Cofactor
PEX5
DTM
DTM
PEX5
DTM
Cytosol
Matrix
or

Figure 1